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Modeling of the Deformation of Living Cells Induced by Atomic Force Microscopy

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ABSTRACT

We describe finite element modeling of the deformation of living cells by atomic force microscopy (AFM). Cells are soft systems, susceptible to large deformations in the course of an AFM measurement. Often the local properties, the subject of the measurement, are obscured by the response of the cell as a whole. The Lagrangian finite deformation model we have developed and implemented in finite elements analysis offers a solution to this problem. The effect of the gross deformation of the cell can be subtracted from the experimentally measured data in order to give a reproducible value for local properties. This facilitates concurrent experimental efforts to measure the mechanical properties at specific receptor sites on the membrane of a living cell.

Keywords: finite element modeling, biological cell, cell membrane, living cell, AFM

1 AFM ON LIVING CELLS

The introduction of new technology to characterize biological systems has been crucial to the current bioscience revolution. One of the current challenges is the development of technology for characterization at the cellular and sub-cellular level. This is needed for the direct study of how genome information is expressed at the microscopic level, for example. Scanning Probe Microscopy has been identified as a promising means to characterize biological systems at scales of nanometers to microns. Atomic Force Microscopy (AFM) and its derivatives such as Recognition Force Microscopy (RFM) are well suited to the characterization of biological systems. [1] AFM uses the deflection of tiny cantilevers in contact with the specimen to provide information about the topography and elastic properties of cells; RFM goes a step further, using molecules attached to an AFM cantilever tip to study the binding at various sites on the specimen. In particular, recent studies have measured the deflection of the AFM cantilever during approach and retraction in order to find the unbinding forces for various ligand-receptor pairs, such as biotin-avidin [2], paired DNA bases [3], antibody-antigen complexes [4] and cell recognition proteins [5]. This paved the way for experiments using a single receptor molecule bound to the AFM tip to map the location of ligands bound to solid surfaces. [6] It is only now becoming possible to use the techniques on living cells, and this has been the aim of our group at Lawrence Livermore National Laboratory. [7]

One challenge with using RFM on living cells is the fact that the cell is not rigid, and as the force is applied to a receptor site, it is not just the receptor site that is affected. The whole cell deforms under the applied force, and the measured binding force is a convolution of the local, intrinsic binding force of the receptor site and the gross elastic response of the cell. We have developed a model of the elastic deformation of the cell in order to separate the two effects.

The model is based on a continuum level analysis of the elastic deformation. [8] The major contributions to the strain energy come from the constitutive response of the incompressible interior, the tension of the membrane and the curvature of the membrane. The membrane in this context refers to a complex structure of multiple phospholipid bilayers and membrane proteins including the cytoskeleton. The curvature energy is known as the Canham-Helfrich Hamiltonian in the statistical mechanics community [9]; the form of the other terms in the energy has been studied in the context of solid mechanics of hyperelastic media. The novel features of this formalism are the treatment of the Canham-Helfrich Hamiltonian for finite deformations and the combination of these energies in the model of a single system.

2 AN ARCHETYPICAL CELL SYSTEM

The bovine sperm cell has been selected as the model system for our experiments to develop the AFM and RFM techniques on living cells. An AFM topographic scan of the cell is shown in Fig. 1. The figure is a perspective view of a single sperm cell generated from an AFM scan in which height is measured in tapping mode as a function of x-y position. This particular scan was obtained in a study by Allen, Bradbury and Balhorn that examined the shape of sperm cells that had been frozen for storage and then thawed in aqueous solution. [10]

The sperm cell was chosen for these experiments be-

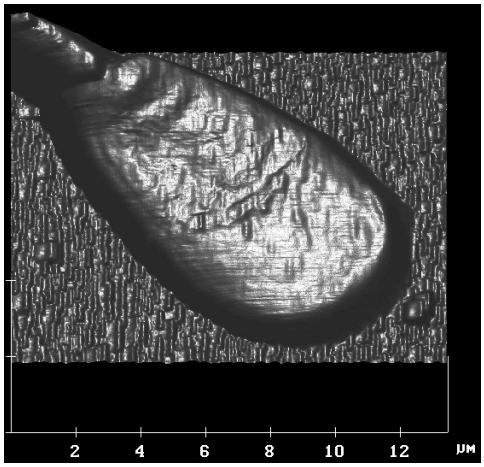

Figure 1: Topography of the bovine spermatazoum from a force-volume scan by atomic force microscopy (AFM). [10] These cells have been selected as our initial test case since they have been well-characterized by other means and they have a well-defined shape. The AFM is used in a liquid cell at room temperature. Force-displacement curves have also been measured. [7] (Courtesy of Balhorn and Allen [10])

cause of its well defined shape, because its structure and composition have been well characterized and because it can be triggered to undergo a transformation in which receptor sites become active on the exterior membrane of the cell. The sperm cell has a relatively simple anatomy. [10] It is well suited for its purpose, to transport DNA for reproduction. Most of the head is packed with DNA wrapped in protein to form chromatin. Roughly half of the volume of the head is occupied with chromatin; the other half is water. The chromatin helps define the cell shape, which is long and flat, roughly 10 microns long by 4 microns wide by 0.5 microns thick. The cell membrane also contributes to the shape. The membrane consists of two or three phospholipid bilayers, depending on the site on the cell. The front of the head, the acrosomal region, consists of three bilayers. The section of the head near the tail, the post-acrosomal region, consists of two bilayers. The only organelles inside the cell are the mitochondria located near the region where the tail joins the head. They provide power to drive the flagellum.

Our ultimate goal is to use AFM and RFM to investigate the properties of specific receptor sites on the cell membrane. These studies are underway, but it is beyond the scope of this Article to describe them in any detail. Nevertheless, it should be clear that if the cell is deformed as a whole when a particular site is probed, then the contribution of the gross deformation must be subtracted in order to find the intrinsic properties of the site. We have constructed a mechanical model of the

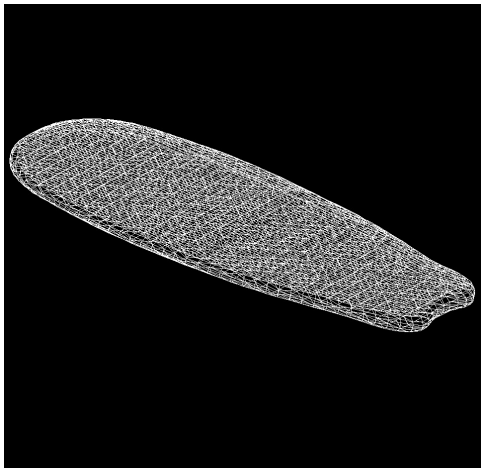

Figure 2: The finite element mesh representation of the cell at its most refined level. Finite element modeling is used to allow analysis of large cell deformations. This avoids many of the assumptions inherent in Hertzian analysis of nanoindentation (see text).

cell, as described below, to allow us to calculate the gross cell deformation induced by the AFM tip. Ultimately, a number of different kinds of AFM measurements will be made to parameterize and validate the model. Most of those experiments are works in progress and will not be described here.

One set of experiments that has been completed is a measurement of the force vs. distance curves during AFM indentation of the cell. [7] These experiments were conducted using a liquid cell AFM in which the sperm cell was immersed in a buffered aqueous solution during the measurements. During indentation and retraction, the displacement of the piezotube that actuates the AFM and the resulting deflection of the AFM cantilever were measured. The force was determined from the cantilever deflection using the known spring constant for the cantilever. The result was a measure of the elastic properties of the cell as a function of the depth of indentation. In the shallow indentation regime the effective stiffness was measured to be 0.03 N/m. [7] This value increased for indentation beyond half of the cell's thickness.

3 FINITE ELEMENT MODEL OF THE DEFORMATION OF BIOLOGICAL CELLS

In an experiment studying nanoindentation of a solid surface, conventional techniques such as Hertzian theory [11] can be employed to extract the elastic constants of the material. This has been employed in the case of cells as well [12], but it has proven problematic because of the large deformations and the finite size of the cell. [13]

Hertzian theory, for example, assumes that the system undergoing indentation is a half-space, a semi-infinite system bounded by a flat surface (before indentation). This is not a good approximation for cells. The radius of curvature of the membrane about the AFM tip is a substantial fraction of the cell's horizontal and vertical dimensions. [7]

Finite element analysis [14] based on a model suitable for large deformations offers a solution to many of the problems of the Hertzian analysis, and it provides the tool needed to subtract off the effects of the gross deformation of the cell. However, neither a suitable analytic deformation model nor an appropriate finite element implementation was available, so both had to be developed. The analytic Lagrangian deformation model we have devised includes both the membrane and the cell interior. There are many examples in the literature where Kirchhoff plate theory has been applied to the membrane [15], [16], but this is not appropriate for a fluid membrane. Our deformation model is based on a Canham-Helfrich strain energy [9] for the membrane

$$w = TJ + AH^2, \tag{1}$$

where T is the surface tension, A is the bending modulus, J is the Jacobian (the area element) and H is the mean curvature (J and H are local measures of stretching and bending, respectively). In addition, an incompressible hyperelastic model was used for the interior. Initially a simple incompressible fluid model was used suitable for the high water content of the interior, but we are in the process of implementing an incompressible neo-Hookian model to capture more of the details of the response of the chromatin package. The shear moduli are expected to be negligible [17] and have not been included. This model is a first attempt to capture the relevant physics of cell deformation with a minimal parameter set. It works well for indentation, but as more data become available it may be necessary to extend the model.

The strain energy yields equations describing cell deformation that are fourth order, non-linear partial differential equations. They are not amenable to analytic solution, but C^1 finite element analysis does provide a means to solve the equations. Initial validation of the model through comparison with force-displacement curves coming from AFM used in a nanoindentation mode is encouraging. The model then allows site-specific mechanical properties to be deconvoluted from the gross cell deformation in RFM experiments.

The current model is suitable for quasi-static deformations of the cell. In many cases it is the viscous properties of the membrane that are of interest. For example, much of the work on biological membranes has studied their fluidity. This affects the rate at which proteins can be transported on the membrane. The finite element model we have developed could be extended to include visco-elastic effects. This is the subject of future investigations.

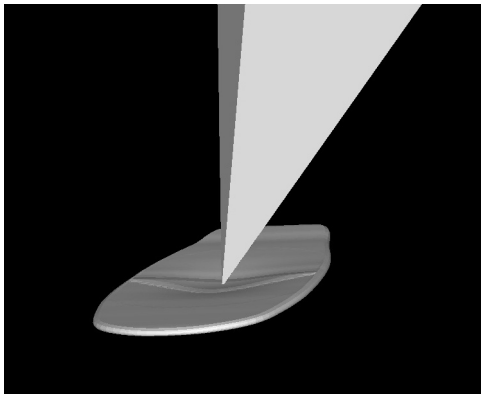

Figure 3: The model geometry of the indented cell and the AFM tip. The length of the cell body and the width of the AFM cantilever are both about 10 μm. The radius of curvature of the membrane about the AFM tip has been computed to be about 1 μm. [7]

4 BEYOND FINITE ELEMENTS

Eventually, it may be possible to use concurrent multiscale modeling to provide a model of the atomistic interactions at the receptor site in addition to the gross deformation of the cell. [18], [19] The principle of multiscale modeling is to use different models in order to describe the physics of the system at different length and time scales. Quantum mechanical electronic structure calculations can be used to calculate chemical bond properties accurately. Classical atomistic models can be used to simulate the deformation of molecules and clusters of atoms. Continuum mechanical models can be used to describe the deformation of macroscopic systems. Each of these effects has an associated length scale, Angstrom, nanometer and micron (or greater), respectively. The emerging paradigm of concurrent multiscale modeling is to describe a heterogeneous system with a hybrid calculation combining different models tuned to different length scales. The different models describe different regions of the system, and they run concurrently in order to achieve self-consistency throughout the simulation. The result is a model that balances accuracy and efficiency, providing a detailed description where it is needed and a simplified description everywhere else.

The RFM system would be an extremely interesting application of concurrent multiscale modeling techniques. The binding of an antibody or ligand to a receptor site could be modeled atomistically, along with the portion of the membrane and solvation layers immediately around the receptor site. This would then be coupled into the continuum description of the rest of the

cell that has been described above. This would allow a very precise study of binding effects that would not need to neglect the contribution of the cell deformation as a whole.

The study of the use of AFM on living cells is just at its genesis. It promises to provide exciting new insight into cellular mechanics at the sub-micron level. Major hurdles remain, both experimentally and computationally, but the initial results are very encouraging.

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